Theor Appl Genet (1994) 89: 964–968

© Springer-Verlag 1994

I. Dweikat · H. Ohm · S. Mackenzie · F. Patterson
S. Cambron · R. Ratcliffe

# Association of a DNA marker with Hessian fly resistance gene *H9* in wheat

Received: 21 February 1994 / Accepted: 28 July 1994

**Abstract** The Hessian fly [*Mayetiola destructor* (Say)] is a major pest of wheat (*Triticum aestivum* L.) and genetic resistance has been used effectively over the past 30 years to protect wheat against serious damage by the fly. To-date, 25 Hessian fly resistance genes, designated *H1* to *H25*, have been identified in wheat. With near-isogenic wheat lines differing for the presence of an individual Hessian fly resistance gene, in conjunction with random amplified polymorphic DNA (RAPD) analysis and denaturing gradient-gel electrophoresis (DGGE), we have identified a DNA marker associated with the *H9* resistance gene. The *H9* gene confers resistance against biotype L of the Hessian fly, the most virulent biotype. The RAPD marker co-segregates with resistance in a segregating $F_2$ population, remains associated with *H9* resistance in a number of different *T. aestivum* and *T. durum* L. genetic backgrounds, and is readily detected by either DGGE or DNA gel-blot hybridization.

**Key words** RAPD · Wheat · Hessian fly · DNA markers

## Introduction

The Hessian fly [*Mayetiola destructor* (Say)] is a destructive insect pest of wheat (*Triticum aestivum* L.) in many parts of the world (Hatchett et al. 1987). Genetic resistance has been used effectively over the past 30 years in the United States to protect wheat against serious damage by the fly. The biological interaction between *Triticum* spp. and the Hessian fly is highly specific. A gene-for-gene relationship has been demonstrated for host resistance and avirulence in the insect (Hatchett and Gallun 1970). To-date, 25 resistance genes, designated *H1* to *H25*, have been effective against the 13 reported biotypes of the Hessian fly (Friebe et al. 1991; Patterson et al. 1992).

Screening for resistance to the Hessian fly requires maintenance of Hessian fly biotypes, a 3-week-period post-infestation for symptom screening, proper incubation conditions, and controlled environment and containment facilities. Consequently, a more rapid, cost-efficient and reliable screening procedure would greatly facilitate the selection process. In addition, a DNA-based screen for Hessian fly resistance would allow the pyramiding of multiple resistance genes, one possible means of enhancing the durability of resistance.

The development of an RFLP map has been much more difficult in wheat than in most other crops due to the polyploid nature of the crop, a high proportion of repetitive DNA, and unusually low levels of DNA polymorphism within the genome (Chao et al. 1989). RFLP analysis was used to tag two Hessian fly resistance genes *H23* and *H24* (Ma et al. 1993). The application of random amplified polymorphic DNA (RAPD) analysis (Williams et al. 1990) has proven useful in plant genotype fingerprinting (Welsh and McClelland 1990; Caetano-Anolles et al. 1991; Hu and Quiros 1991; Yang and Quiros 1993), population and pedigree analyses (Van Heusden and Bachmann 1992; Dweikat et al. 1993), and the detection of DNA polymorphism levels appropriate for the development of DNA markers in a number of self-pollinating crops. The combination of near-isogenic lines and RAPD analysis was used in tomato (Martin et al. 1991), lettuce (Paran et al. 1991), oat (Penner et al. 1993) and common bean (Haley et al. 1993; Miklas et al. 1993) for the purpose of identifying DNA markers for disease resistance genes.

Levels of polymorphism in wheat detected by RAPD analysis using agarose-gel electrophoresis are similar to those detected by RFLP analysis (Devos and Gale 1992). However, fractionation of RAPD products by denaturing gradient-gel electrophoresis (DGGE) significantly enhances the detection of DNA polymorphism in wheat (He

Communicated by P. L. Pfahler

I. Dweikat (✉) · H. Ohm · S. Mackenzie · F. Patterson
Department of Agronomy, Purdue University, West Lafayette, IN 47907, USA

S. Cambron · R. Ratcliffe
Department of Entomology and USDA-ARS, Purdue University, West Lafayette, IN 47907, USA

Purdue University, Agric. Exp. Stn. Journal paper No. 14440

et al. 1992; Dweikat et al. 1993). The purpose of the present study is to take advantage of RAPD-DGGE to identify DNA markers closely linked to the Hessian fly resistance gene *H9*, which confers resistance to biotype L of the Hessian fly, a prevalent biotype in the field in eastern USA.

## Materials and methods

### Plant materials

The plant materials used for this study consisted of *T. aestivum* L. cultivar Newton, CI 17715, and four near-isogenic lines (NILs) of wheat differing for the presence of single genes that confer Hessian fly resistance (Patterson et al. 1994): *T. aestivum* line Ella, CI 17938; *T. aestivum* line 86975; *Triticum turgidum* L. line D6647, CI 15329; and *T. turgidum* line IN8455. All four NILs, containing genes *H3*, *H5*, *H6* or *H9*, were developed by five or six cycles of backcrossing to Newton with selection for resistance (Patterson et al. 1994). Newton is a hard winter wheat susceptible to all known biotypes of the Hessian fly. Gene *H9* was transferred from the source line Elva (*T. turgidum*) to line Ella (Patterson et al. 1982). *H9* was subsequently transferred from Ella to soft red winter wheat lines by five cycles of backcrossing and selection, to produce line 86975. *H9* was transferred from Ella to D6647 by three cycles of backcrossing and selection to produce line IN8455 (D6647*4/Ella). D6647 is susceptible to biotype L of the Hessian fly and does not contain *H9*. An $F_2$ population of 126 individuals was derived from the cross Newton //Newton*7/*H9*.

### DNA isolation, PCR reactions, gel electrophoresis

DNA was isolated from 1 g of fresh tissue from 2-week-old near-isogenic parental lines and $F_2$ seedlings using a modified CTAB extraction method (Dweikat et al. 1993). Oligonucleotide primers (10-mers) were purchased from Operon Technologies (Alameda, Calif.) and from the University of British Columbia (Vancouver, Canada). The PCR reaction conditions reported by Williams et al. (1990) were followed with minor modification. Reaction mixtures (50 µl total volume) consisted of 10 mM of Tris-HCl, pH 9.0 at 25°C, 50 mM of KCl, 1.8 mM $MgCl_2$, nucleotides dATP, dTTP, dCTP, and dGTP (200 mM each), 0.2 mM of primer, 100 ng of template DNA, and 2.0 units of *Taq* DNA polymerase (Promega). When two primers were used together, a concentration of 0.2 M of each was used. The reaction mixture was overlain with 50 µl of light mineral oil (Sigma Chemical Co.). Amplifications were carried out in a MJ Research PTC-100 thermocycler programmed for 40 cycles of 1 min at 94°C, 50 s at 36°C, 1 min at 72°C, and ending with 6 min at 72°C. The primers were used singly and in pairwise combinations.

The PCR products were fractionated by denaturing gradient-gel electrophoresis with a denaturant gradient range of 10–50% (Dweikat et al. 1993).

### DNA gel blot hybridization

Total genomic DNA (10 µg) was digested with *Eco*RI and *Eco*RV restriction endonucleases (Promega) according to the manufacturer's recommendations, subjected to electrophoresis through 0.8% agarose for 18–20 h at 2.4 V/cm, and blotted to Magnagraph membrane (MSI) following the manufacturer's recommendations. The polymorphic RAPD-derived DNA segment associated with the *H9* gene was excised from the gel and heated at 94°C in 100 µl of TE, pH 7.5, for 5 min. One microliter of the sample was used as template to re-amplify the polymorphic fragment using the same PCR conditions described above. A 50-ng sample of the re-amplified fragment was labelled with $^{32}$P-dCTP by the random primer method (Feinberg and Vogelstein 1984). Pre-hybridization and hybridization conditions were according to the membrane manufacturer's recommendations.

### Evaluations of resistance

$F_2$ plants, from which DNA was extracted, were selfed to produce $F_3$ seeds. Resistance in $F_3$ families to Hessian fly biotype L was evaluated as described by Cartwright and LaHue (1944). Seedlings (14–15) were grown in ten rows in wooden flats 54↔36↔8 cm. Nine progeny rows of 15 seeds per row and two half-rows of ten seeds of parent lines (controls) were planted per flat. Each row contained the progeny of a single $F_2$ plant. Seedlings were grown to the one-leaf stage in a growth chamber at 20°C and then infested with biotype L of the Hessian fly. The level of infestation (number of live larvae/seedling) was estimated to be between 8–10 larvae/seedling. About 3 weeks after infestation, progeny rows were classified as resistant, segregating, or susceptible.

## Results

We surveyed 650 single primers and 190 two-primer combinations against Newton and four NILs containing the resistance genes *H3*, *H5*, *H6*, and *H9*. We observed one DNA polymorphism associated with *H9* after screening 450 primers. Only by fractionating the RAPD products by DGGE with a denaturant gradient of 10–50% were we able to detect the polymorphism (Fig. 1A, B). The DNA polymorphism observed was a result of combining primers OpA09 and OpA17. Neither primer produced a polymorphism associated with *H9* when used singly (Fig. 2). DNA

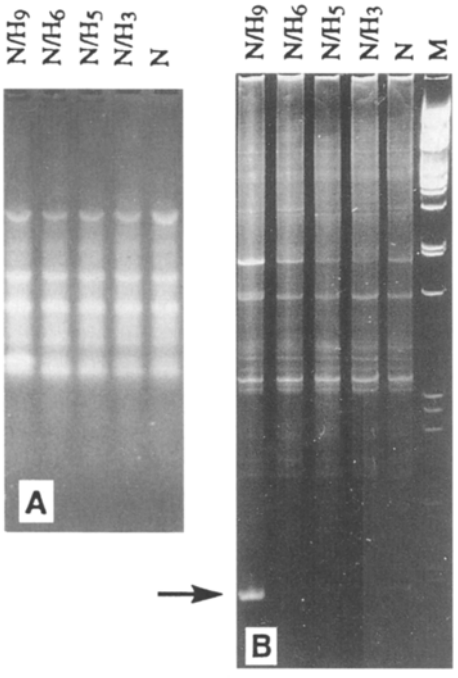


**Fig. 1A, B** Comparison between DGGE and agarose-gel electrophoresis in resolving a polymorphism associated with gene *H9*. **A** PCR amplification of wheat genomic DNA using random oligonucleotide primers OpA09/OpA17 and fractionated in 1.2% agarose, 0.5 x TBE. **B** PCR amplified products identical to those in **A** but fractionated by denaturing gradient-gel electrophoresis. Newton (*N*), a susceptible line, is shown with four NILs, each containing a different gene for Hessian fly resistance (*M* designates *Pst*I-digested lambda MWt markers). The *arrow* indicates the presence of a fragment polymorphism associated with *H9*

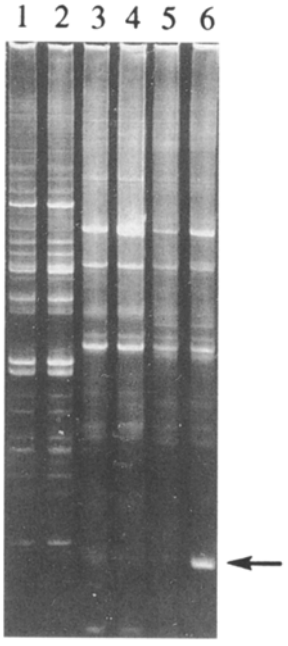


**Fig. 2** PCR amplification of genomic DNA from Newton (*lanes 1, 3, and 5*), and a *H9* NIL (*lanes 2, 4, and 6*). *Lanes 1 and 2* demonstrate the results of amplification using a combination of primer OpA9 and OpA17 (0.2 μM each). *Lanes 3 and 4* demonstrate the results of amplification using primer OpA9, while *lanes 5 and 6* are samples amplified using OpA17

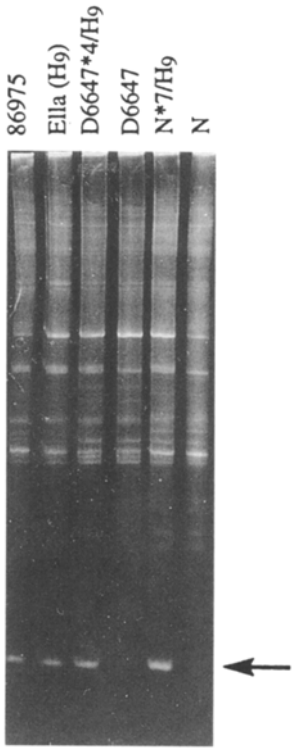


**Fig. 4** PCR amplification of wheat total genomic DNA fractionated by DGGE. Lines Newton (*N*), a hard winter wheat cultivar (*T. aestivum*), and tetraploid *T. turgidum* line D6647 (*T. durum*), lack the *H9* gene. N*7/*H9* (Iris) and D6647*4/H9 (IN8455) both contain the *H9* gene. Ella is the donor of gene *H9* in 86975. Line 86975 is a soft winter wheat (*T. aestivum*) that contains *H9*. The *arrow* indicates the DNA polymorphism associated with *H9* and demonstrated in Fig. 1B

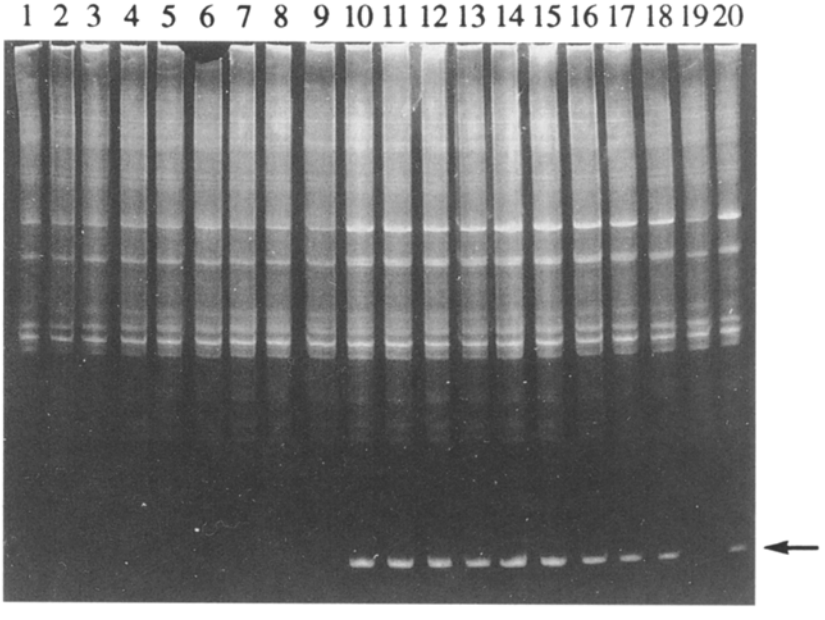


**Fig. 3** PCR amplification of genomic DNA from 18 $F_2$ seedlings derived from the cross Newton //Newton*7/*H9* and segregating for gene *H9*. *Lanes 1–9* represent susceptible seedlings, *lanes 10–18* represent resistant seedlings. *Lanes 19 and 20* represent the susceptible parent (Newton) and resistant parent (Newton*7/*H9*), respectively. The *arrow* indicates a polymorphic band associated with *H9*

polymorphisms were also observed in association with genes *H3*, *H5* and *H6* (manuscript in preparation).

To verify an association between the DNA polymorphism and the resistance gene *H9*, these two primers were then used to amplify genomic DNA from 126 $F_2$ seedlings produced from the cross Newton*7/Ella (our source of the *H9* gene) //Newton. Figure 3 illustrates results from this experiment. The identified RAPD marker co-segregated with the *H9* resistance gene, demonstrating linkage in the coupling phase, with no evidence of recombination between the marker and the resistance gene. The $F_2$ plant classifications for resistance were verified by testing 15 $F_3$ progenies from individual $F_2$ plants against Hessian fly biotype L. The $F_3$ families segregated for a reaction to the Hessian fly in a ratio of 28 homozygous resistant : 68 heterozygous resistant : 30 homozygous susceptible, producing a reasonable fit to a 1:2:1 ratio ($\chi^2$=0.86). Of 126 progeny, all 96 resistant plants exhibited the polymorphic band while the remaining 30 susceptible plants did not.

For any DNA marker to be useful in a marker-assisted selection program, different genetic backgrounds must not interfere with the detection of the linked DNA marker. To

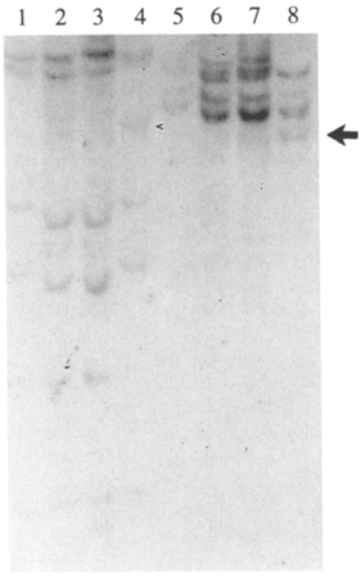


**Fig. 5** Total genomic DNA from susceptible Newton (*lanes 1 and 5*), Newton-*H3* (*lanes 2 and 6*), Newton-*H6* (*lanes 3 and 7*)and Newton-*H9* (*lanes 4 and 8*) digested with *Eco*RV (*lanes 1–4*) or *Eco*RI (*lanes 5–8*), blotted and hybridized with a PCR-amplified polymorphic DNA fragment associated with *H9* (see Fig. 1B). The *large arrow* indicates an *Eco*RI-generated DNA polymorphism associated with *H9;* the *small arrow* indicates an *Eco*RV-generated DNA polymorphism associated with *H9*. Some apparent band shifts in *lanes 2, 3, and 5* are the result of unequal sample loading per lane

test for genetic background effects on the detection of the *H9*-associated RAPD marker, and to verify that the linkage between this marker and the *H9* gene would be retained over several backcrosses, we tested four different lines containing *H9*, two derived from *T. durum* and two from *T. aestivum*. All lines containing the *H9* gene (verified by testing against biotype L) contained the marker (Fig. 4).

To use the identified RAPD markers for future high-resolution mapping studies, it is important to examine the relative copy number of this DNA marker in the wheat genome. When the polymorphic band was extracted from the gel, radioactively labelled, and used as a probe on DNA gel-blots containing digested genomic DNA from the NILs, the probe hybridized to a low-copy or single-copy sequence in the genome. Most importantly, a DNA polymorphism associated only with *H9* was detected (Fig. 5).

## Discussion

The use of NILs for locating genes of interest in wheat provides an efficient means of identifying those polymorphic sites that have the highest probability of being located close to the gene of interest (Young et al. 1988; Martin et al. 1991; Haley et al. 1993; Miklas et al. 1993). The combined RAPD-DGGE system has proven useful in detecting higher levels of DNA polymorphism in wheat than can be detected by fractionating PCR products with agarose-gel electrophoresis (He et al. 1992; Dweikat et al. 1993). Based on previous observations, we took advantage of this system for our study. An average of 12 loci (scorable RAPD bands) per primer were observed by DGGE compared to eight loci on agarose gels. The combination of two primers, in most cases, generates a different DNA pattern from those obtained with each single primer (Klein-Lankhorst et al. 1991; Dweikat et al. 1993). This increases the analytical power of this procedure by increasing the number of amplifiable products from a limited number of primers.

Hessian fly resistance genes *H3*, *H6*, and *H9* are all located on chromosome 5A in wheat within a linkage block spanning about 15 map units (Stebbins et al. 1982). The *H5* gene is located on chromosome 1A (Roberts and Gallun 1984). The NILs for *H3*, *H6*, and *H5* were included in the screening process because all of these lines were developed by backcrossing to the same recurrent parent 'Newton'. This common genetic background facilitates the detection of a polymorphism associated with any of these genes and, at the same time, facilitates the identification of those polymorphisms that are gene-specific, i.e., associated with *H9* only and not with the linked *H6* or *H3* genes. We are continuing efforts to identify multiple markers associated with each of these genes. Our goal is to establish a high-resolution map for the region of chromosome 5A encompassing *H3*, *H6*, and *H9*. Concurrently, we intend to verify tight linkage between identified RAPD markers and the genes of interest for the purposes of facilitating a marker-assisted selection strategy for Hessian fly resistance in wheat. The importance of the present study was in demonstrating the relative ease with which a particular gene within the wheat genome can be targeted for marker identification. This has not been the case in wheat to-date. With this study, we have demonstrated that it is feasible to identify tightly linked RAPD markers in wheat associated with genes of interest. The marker linked to H9 remained associated with it even after several generations of backcrossing into recipient lines, demonstrating the usefulness of this marker for the transfer of *H9*. This procedure will also clearly be appropriate to identify low-copy sequences necessary for high-resolution mapping in the extraordinarily large *Triticum* genome.

## References

Caetano-Anolles G, Bassam BJ, Gresshoff PM (1991) DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Biotechnology 9:553–557

Cartwright W, LaHue D (1944)Testing wheats in the greenhouse for Hessian fly resistance. J Econ Entomol 37:385–387

Chao S, Sharp P, Worland A, Warham E, Koebner R, Gale MD (1989) RFLP-based genetic maps of wheat homoeologous group-7 chromosomes. Theor Appl Genet 78:495–504

Devos KM, Gale MD (1992) The use of random amplified polymorphic DNA markers in wheat. Theor Appl Genet 84:567–572

Dweikat IM, Mackenzie S, Levy M, Ohm H (1993) Pedigree assessment using RAPD-DGGE in cereal crop species. Theor Appl Genet 85:497–505

Feinberg AP, Vogelstein B (1984) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 137:266–267

Friebe B, Hatchett J, Gill B, Mukai Y, Sebesta E (1991) Transfer of Hessian fly resistance from rye to wheat via radiation-induced terminal and intercalary chromosomal translocations. Theor Appl Genet 83:33–40

Haley SD, Miklas PN, Stavely JR, Byrum J, Kelly JD (1993) Identification of RAPD markers linked to a major rust resistance gene block in common bean. Theor Appl Genet 86:505–512

Hatchett J , Gallun R (1970) Genetics of the the ability of the Hessian fly, *Mayetiola destructor*, to survive on wheat having different genes for resistance. Ann Entomol Soc Am 63:1400–1407

Hatchett J, Starks K, Webster J (1987) Insect and mite pests of wheat. In: Heyne EG (ed) Wheat and wheat improvement, 2nd edn. Am Soc Agron, Madison, Wisconsin, pp 625–675

He S, Ohm AH, Mackenzie S (1992) Detection of DNA sequence polymorphism among wheat varieties. Theor Appl Genet 84:573–578

Hu J, Quiros C (1991) Identification of broccoli and cauliflower cultivars with RAPD markers. Plant Cell Rep 10:505–511

Klein-Lankhorst RM, Vermunt A, Weide R, Liharsha T, Zabel P (1991) Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). Theor Appl Genet 83:108–114

Ma Z-Q, Gill BS, Sorrells ME, Tanksley S (1993) RFLP markers linked to two Hessian fly resistance genes in wheat (*Triticum aestivum* L.) from *Triticum tauschii* (Coss) Schmal. Theor Appl Genet 85:750–754

Martin G, Williams J, Tanksley S (1991) Rapid identification of markers linked to a *Pseudomonas* resistance gene in tomato by using random primers and near-isogenic lines. Proc Natl Acad Sci USA 88:2336–2340

Miklas PN, Stavely JR, Kelly JD (1993) Identification and potential use of a molecular marker for rust resistance in common bean. Theor Appl Genet 85:745–749

Paran I, Kesseli R, Michelmore R (1991) Identification of restriction fragment length polymorphism and random amplified polymorphic DNA markers linked to downy mildew resistance genes in lettuce, using near-isogenic lines. Genome 34:1021–1027

Patterson, F, Gallun RL, Stebbins NB, Carlson SK (1982) Registration of Stella and Ella common wheat germplasm lines. Crop Sci 22:902–903

Patterson F, Foster J, Ohm H, Hatchett J, Taylor P (1992) Proposed system of nomenclature for biotypes of Hessian fly (Diptera: Cecidomyiidae) in North America. J Econ Entomol 85:307–311

Patterson F, Mass F, Foster J, Ratcliffe R, Cambron S, Safranski G, Taylor P, Ohm H (1994) Registration of eight Hessian fly-resistant common winter wheat germplasm lines (Carol, Erin, Flynn, Iris, Joy, Karen, Lola and Molly). Crop Sci 34:315–316

Penner GA, Chong J, Levesque-Lemay M, Molnar SJ, Fedak G (1993) Identification of a RAPD marker linked to the oat stem rust gene *Pg3*. Theor Appl Genet :702–705

Roberts J, Gallun R (1984) Chromosome location of the *H5* gene for the resistance to Hessian fly in wheat. J Hered 75:147–148

Stebbins N, Patterson F, Gallun R (1982) Interrelationships among wheat genes *H3*, *H6*, *H9*, and *H10* for Hessian fly resistance. Crop Sci 22:1029–1032

Van Heusden AW, Bachmann K (1992) Genotype relationships in *Microseris-Elegans* (Asteraceae, Lactuceae) revealed by DNA amplification from arbitrary primers(RAPDs). Plant Syst Evol 179:221–233

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18:7213–7218

Williams J, Kubelik A, Livak K, Rafalski J, Tingey S (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18:6531–6535

Yang X, Quiros C (1993) Identification and classification of celery cultivars with RAPD markers. Theor Appl Genet 86:205–212

Young N, Zamir D, Ganal M, Tanksley S (1988) Use of isogenic lines and simultaneous probing to identify DNA markers tightly linked to the *Tm-2a* gene in tomato. Genetics 120:579–585